Microorganisms for wheat improvement under biotic stress and dry climate

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Abstract: Wheat is the most important grain crop, a main sustenance for a third of the world population. Biotic stress by diseases represents a serious risk to wheat production particularly under dry condition. In this work the use of plant beneficial microorganisms as bio elicitors to improve the productivity of two common wheat varieties (i.e. Gemmiza 10 and Sakha 93) were achieved. The Bio elicitors used are *Streptomyces griseus, Trichoderma harzianum, T. viride, Rhodotorula glutinis*, *Paenibacillus polymyxa, Bacillus subtilis, Pseudomonas putida*, *P. fluorescens* reduced wheat diseases occurrence including rust, net blotches and powdery mildew in comparison with natural elicitors, as methyl jasmonate, chitosan, ascorbic acid and Putrescine grown in new reclaimed areas in Sinai and arable land in Behira Governorate. Results show that bio elicitors enhanced and increase total phenols (mg catechol/g F.W.), hormonal stress as peroxidase activity (U g^{-1} of fresh weight), chitinase (nKat/mg^{-1} of Protein) and total soluble protein (µg/mg FW) in both varieties as well as promote growth rate, yield, flour starch, protein contains and gluten in both wheat grains verities. The obtained data show that the used microorganisms have enhanced and improved the wheat tolerance to biotic stress. That innovation might make advantageous in alleviating effects of environmental changes on other crops and expanding the wheat cultivation in the new reclaimed lands under arid climate condition.

Keywords: Bio elicitors, biotic stress, dry climate, wheat

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1 Introduction

Wheat (*Triticum aestivum* L.) is the most important grain crop in all over the world. In Egypt, wheat total cultivated area is 3.2 million feddan (1 feddan = 4200 m^2) and 9.5 million ton average production (Hasan, 2013). At the same time, diseases as rusts, powdery mildew, spots represent the main biotic stress that reduce wheat production (Haggag and Abd-El-Kareem 2009; Haggag, 2013). Wheat leaf rust caused by *Puccinia triticina*, blotch (*Pyrenophora triticirepentis*) and septoria complex (*Septoria* spp.) are the common diseases in the new cultivated area (Agrios, 2005). The fungal *Puccinia hordei* Otth is an economically important disease in most of temperate regions, which could largely decrease the yield of susceptible cultivars up to about 60% and reduces grain quality. Pathogens threats are measure by complex changes in crops and agricultural practice that may result from climate change (Butterworth et al., 2010).

Key of disease management issues should be address for refining and increase food security under climate change (Pautasso et al., 2012; Haggag, 2016). Innovative approaches in plant diseases management is required for given an increased importance of altered agroecosystems (Haggag et al. 2014; Newton et al., 2011). Bio elicitors associated with plant roots are known to protect, promote plant growth, induce systemic resistance of plant to stress include signaling molecules such as salicylic acid and jasmonic acid as reported by (Walters et al., 2002; Haggag, 2005; Haggag and Abd-El Khair, 2007; Holopainenauer et al., 2009). The rhizobacterium *Pseudomonas spp.* have ability to grow under

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low-nutrient conditions and induce systemic resistance against plant stress (Haggag, 2013, Haggag et al., 2013). *Pseudomonas spp.*, *Streptomyces spp.* and *Bacillus spp.* could colonise the roots of crop plants and produce metabolites represent a real alternative to the application of chemical fungicides. The aim of this study is to investigate the effect of different bio elicitors to improve the tolerance of wheat against biotic stress including leaf rust, blotch and septoria diseases under aridity condition of new reclaimed areas in Sinai compared with arable land in Behira Governorate.

2 Materials and Methods

2.1 Field experiments

Two field experiments were implemented for wheat cultivation using Gemmiza 10 and Sakha 93 varieties. The first field is located in North Sinai, while the second is situated in Behira Governorate as shown in Figure 1. Experimental design was randomized complete block design with five replicates and 10.5 m^2 experimental unit. Ten rows of wheat seeds were sown with a density of 500 seeds per square meter. All plots were fertilized immediately after sowing with ammonium nitrate (NH_4NO_3) at the rate of 100 $kg{\cdot}N{\cdot}ha^{-1}$. Wheat seeds were surface-sterilized, treated with the selected bioagents. Liquid formulations were sprayed, especially, on the wheat leaves at 30 and 60 days after sowing. In this study, 10^7 cfu mL^{-1} of bacterial cell suspension and 10^5 cfu mL^{-1} of fungal cell suspension were used. Two months later, 10 individual plant samples from each experimental unit were carefully harvested an adhering soil removed by washing. Fungal infection of roots was evaluated and fresh weight and dry weight (70°C for 72 h) were determined.

2.2 Climate

The data recorded by the Central Laboratory for Agricultural Climate (CLAC, 2015) indicate that the average of air temperature in North Sinai ranges between 13.6°C and 27.0°C while it varied from 12.4°C to 26.6°C In Behira Governorate. Low values of air temperature are recognized during December - February period, while the high values associated with June - September interval in both areas. Rainfall reaches a total of 103.9 and 56.9 mm per year in the experimental fields of Sinai and Behira respectively. Usually, dry season stretched from June to September interval in North Sinai, while extended from May to September in Behira as shown in Figures 2 and 3. Averages of relative humidity (%), wind speed (Node), and sunshine (hour) are 70.5,4.5 and 8.4 in North Sinai, while attained 63.9, 4.4 and 9.0 in Behira Governorate respectively. These data indicate that the experimental fields are characterized by exact dry condition. According to USDA-NRCS (2010) and the above mentioned data the soil moisture regime is *Torric* while the temperature regime is *Thermic*.

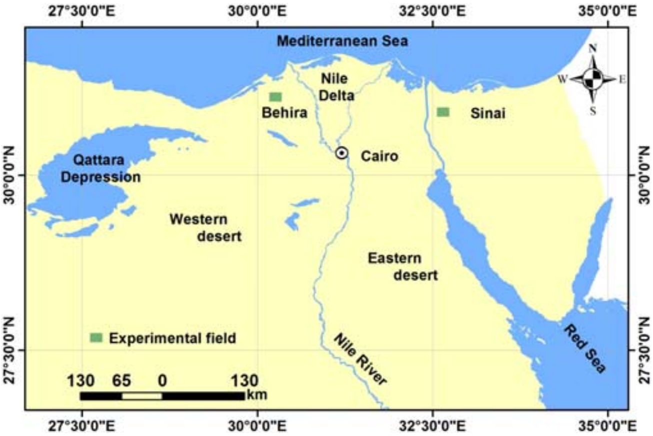

Figure 1 Location of the experimental fields

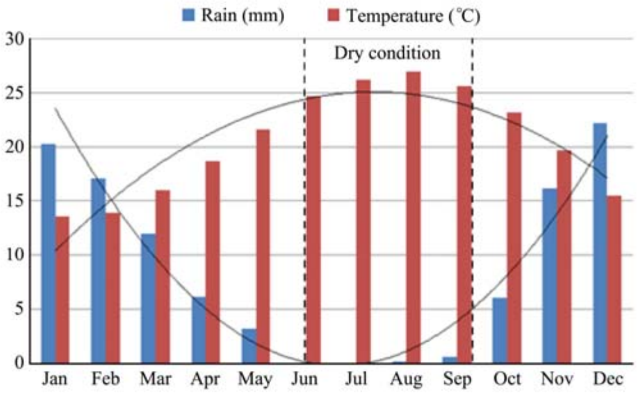

Figure 2 Air temperature and rainfall averages (2015) of North Sinai

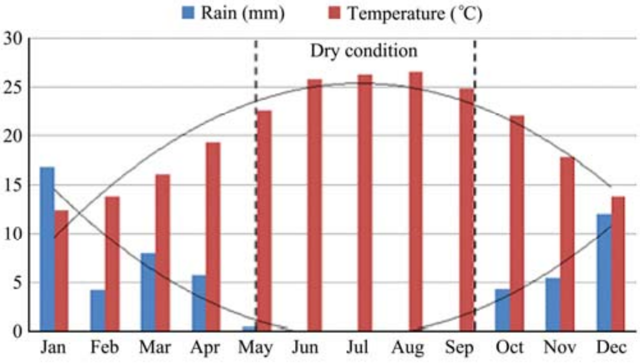

Figure 3 Air temperature and rainfall averages (2015) of Behira Governorate

2.3 Microorganisms

Isolates of the bio elicitors, *Streptomyces griseus, Trichoderma harzianum, T. viride, Rhodotorula glutinis, Paenibacillus polymyxa, Bacillus subtilis, Pseudomonas putida* and *P. fluorescens* isolated from healthy wheat plants were used as bio-elicitors in this study in compare with natural elicitors as methyl jasmonate, chitosan, ascorbic acid and putrescein.

The fungus and the bacterial isolates were routinely cultured on Potato Dextrose, nutrients and King's Medium B (KMB), respectively. Isolates density were standardized by adjusting it to approximately 6×10^5 and 6×10^7 cfu mL^{-1} for fungus and bacteria respectively. Isolates were previously screening for their abilities to grow in medium supplemented with different concentrations of NaCl (0.2 to 1.8 M) as well as temperature at 25°C, 30°C, 35°C, 40°C and 45°C (Haggag et al., 2016).

2.4 Leaf rust

Disease incidence, i.e. per cent of disease-affected leaves (P) was calculated according to the formula: n ─ P = N·100, where n – number of affected leaves, N – number of assessed leaves. The disease-affected leaf area was estimated in per cent according to the scale recommended by the European Plant Protection Organisation (EPPO). This scale is included in the EPPO Standards (1997). Disease severity (R) was calculated according to the formula, having added per cent of affected leaf area of each leaf and having divided the sum by the number of assessed leaves: ∑(n·b) ———, R = N where ∑(n·b) – sum of product of the number of leaves with the same percent of severity and value of severity, N – number of assessed leaves.

2.5 Powdery mildew

Leaves colonization by the fungus was quantified by measuring mildew colonies covering the surface of the leaves. Ten days after inoculation, disease severity was recorded according to the following scales: 0 = 0%; 1 ≤ 5%; 3 = 6%-15%; 5 = 16%-25%; 7 = 26%-50%; and 9 ≥ 50% (Leath and Heun, 1998). Leaf spots were measured by measuring spots colonies covering the surface of the leaves.

2.6 Soil and water analyses

Soil and water samples were collected according to FAO (2006) guidelines to represent the experimental fields. Laboratory analyses for representative samples were carried out following the methods detailed by Soil Survey Staff (2014).

2.7 Plant analysis

Two months after sowing, ten leaves per plant were separately collected, frozen for 36 h, dried and powdered. Generally, 100 mg dried sample were used for analysis.

- Total phenols: Free and conjugated phenols were determined in treated leaves, 15 days after plant spraying with chemical elicitors according to (AOAC, 1975) using the Folin–Danis reagent. Total phenols were identified by HPLC using a reverse phase C8 column and compared with a catechol standard (Sigma chemicals). Hormonal stress as peroxidase activity and chitinase were also determined. The reaction mixture contained 0.1 mmol EDTA, 1 mL of 0.2 mol/m^3potassium phosphate buffer with pH = 7.6, 0.1 mL of 2 m mol (NADPH), 0.5 mL of 3 m mol DTNB, 0.1 mL enzyme extract. The raise within absorbance at 412 nm was recorded at 25°C in excess of a period of 5 min on a spectrophotometer. Chitinase was assayed using 3, 5-dinitrosalicylic acid reagent to determine the free aldehydic groups of hexoaminase liberated on chitin digestion according to the method described by (Ishaaya and Casida 1974).

- Total soluble protein: Soluble protein extraction was carried out according to Bollag and Eldelstein (1992).

2.8 Grain quality tests

All the plants of different treatments were harvested and data on wheat grain yield was recorded. The flour yield is of importance to the miller because it is a general indication of how much flour can be made from a given grain. It was conducted on a laboratory mill (e.g., Buhler or Miag Multomat) under a standard operating procedure. The value obtained (*i.e.* the percentage of flour based on the initial weight of wheat) relates to the extraction rate on a commercial mill. Gluten content was determined on 10 g sample by washing with a 2% NaCl solution buffered at pH 6.8 (AACC, 2000). The extracted wet gluten was dried in a heated plate, weighed and the result was adjusted on dry matter basis.

2.9 Statistical analysis

Results were analyzed using an ANOVA of squaretransformed data. Significant differences were assessed by comparison the differences between means using Least Significant Difference (LSD) value at 0.05.

3 Results

3.1 Soils and Water

Analyses of soils and water samples of the experimental sites indicate that the experimental fields are characterized by sandy soil texture, soil salinity (EC) of 7.12 dS/m in Saini and 1.72 dS/m in Behira fields, soil pH reach 7.7 in Sinai site and 7.9 in Behira. The dominant salts in the soils of experimental field are NaCl and $CaCl_2$. Analyses of irrigation water indicate that the water salinity (EC) is high in Sinai experimental field (8.01 dS $m^{-1)}$, while it is low in Behira (1.9 dS m^{-1}). The values of water pH are 7.5 in North Sinai and 7.8 in Behira. In view of the Keys to Soil Taxonomy detailed by USDA-NRCS (2010) the investigated soils were classified as *Typic Torripsamment*.

3.2 Evaluation of bio-elicitors

Effective of different bio and natural elicitors on controlling of foliar diseases of two wheat varieties i.e. cv. Gemmiza 10 and Sakha 93 were evaluated in two different environmental conditions i.e. new reclaimed areas in Sinai and arable land in Behira during 2015, 2016 and 2017 seasons. Data showed that the all diseases incidence were higher in untreated wheat plants in both wheat varieties i.e. cv. Gemmiza 10 and Sakha 93, regions and seasons. Powdery mildew, leaf rust and leaf spots are the most important and theater diseases of wheat that cause severe infection, respectively in both varieties (Tables 1 and 2). Significant differences of diseases incidence were recorded among treatment, regions, cultivars and untreated control. Wheat variety Sakha 93 cv. is more resistant to all diseases than Gemmiza cv. Powdery mildew and leaf rust are the most important that cause severe losses in Behira Governorate, meanwhile leaf spots causes severe loss in Sinai (Tables 1 and 2). Our experiments showed that under stress condition, bio-elicitors reduced diseases incidence significantly, whereas *Pseudomonas sp.* and *P. polymyxa* showed significant potential against all diseases in both regions and seasons (Tables 1 and 2). *Rhodotorula glutinis* was more effective against spots in both wheat varieties and seasons. Analysis of data indicated that all compounds treatments significantly reduced disease severity under normal and saline conditions in both wheat varieties. Significant differences were obtained among treatments and untreated control. Our results clearly show that the chitosan and methyl jasmonate markedly enhanced resistance to diseases control. The control plant developed a strong disease severity. This may be to increase plant defense compounds against pathogens. In salt soil, most of the tested isolates showed good effects.

Table 1 Diseases severity of wheat plants cv. Sakha 93 grown under dry condition of Sinai and Behira

Treatments	Powdery mildew				Leaf rust				Leaf Spots			
	Sinai		Behira		Sinai		Behira		Sinai		Behira	
	Season* I	Season** II	Season I	Season II	Season I	Season II	Season I	Season II	Season I	Season II	Season I	Season II
Streptomyces griseus	2.3	1.2	1.6	1.3	1.8	1.9	2.2	2.5	3.2	2.4	3.4	3.4
Bacillus subtilis	1.3	1	1.3	1.6	1.2	1.3	1.9	1.4	3.6	3.5	2.6	3.6
Paenibacillus polymyxa	1.8	1.6	1.8	0.3	1.2	1.3	1.3	1	1.8	1.4	1.6	1.3
Pseudomonas putida	1.6	1.2	1.3	9.4	1.3	1.4	1	1.6	1.9	1.3	1.3	1.3
P. fluorescens	1.2	1	2.4	2	2.6	2.6	2.5	2	2.2	1.4	2.3	1.6
Rhodotorula glutinis	2	2	2.1	2	2	2.2	2.3	2.4	1.1	1.6	1.6	1.8
Trichoderma harzianum	2.1	2.4	2.2	2.3	1.7	1.8	2.8	2.5	1.1	1.4	1.9	1.6
T. viride	2.3	1.8	2.6	1.6	1.9	1.8	2.1	2.6	1.4	2.4	1.8	3.4
Methyl jasmonate	2.4	1.7	2.7	2.8	1.8	1.9	3.2	2.6	2.3	4.9	3.9	3.9
Chitosan	3.8	3.2	3.8	3.6	2.1	2.2	3.2	2.7	4.9	2.5	3.2	2.3
Ascorbic acid	4.3	3.2	3.9	3.3	5.2	6.2	5.4	5.3	2.6	3.3	3.1	3
Putrescine	2.4	0.2	1.8	3.22	4.4	4.5	4.7	4.36	1.6	0.53	3.5	3.5
Untreated control	10.3	9.7	18.2	23.7	14.1	15.1	19.6	18.3	17.2	18.2	14.1	13.2
LSD	0.9	0.7	0.22	0.8	0.7	0.72	0.43	0.3	0.63	0.5	0.4	0.5

Note: *Season I: 2015 and 2016 season; ** season II: 2016 and 2017 season.

Table 2 Diseases severity of wheat plants cv. Gemaza 10 grown under dry condition of Sinai and Behira

Treatments	Powdery mildew				Leaf rust				Leaf Spots			
	Sinai		Behira		Sinai		Behira		Sinai		Behira	
	Season I	Season II	Season I	Season II	Season I	Season II	Season I	Season II	Season I	Season II	Season I	Season II
Streptomyces griseus	1.3	1.1	1.4	1.4	0.4	0.3	2.7	2.6	2.7	2.3	2.4	2.3
Bacillus subtilis	1.1	1	1.5	1.4	0.5	0.3	2.8	2.6	3.7	3.2	2.3	2.2
Paenibacillus polymyxa	1.6	1.3	3.2	2.2	0.8	0.9	2	2	2	2	1.1	1.3
Pseudomonas putida	1.1	1.1	1.7	1.6	0.8	7.3	0.6	2.3	2.2	2.3	1.2	1.3
P. fluorescens	1.6	1.3	3.2	2	0.5	0.9	2.2	2.4	2.6	2.3	2.1	2.3
Rhodotorula glutinis	1.3	1.2	2.8	2.3	0.8	1.2	2.5	2	3.6	3.4	3.4	3
Trichoderma harzianum	1.9	1.3	2.7	2.1	1.1	1	2.1	2	1.7	1.2	3.5	3
T. viride	1.7	1.3	2.2	2.3	2.2	2.2	2.2	2.2	1.6	1.7	3.1	3
Methyl jasmonate	1.7	2.6	2.5	2.6	2.6	1.4	3.2	4.4	3.2	3.9	3.2	4.9
Chitosan	2.7	2	5.7	3.3	2.2	2.6	3	3.3	2.9	3.3	4.5	3
Ascorbic acid	2.3	2	3.2	4.3	2.4	2.3	3.1	3.3	2.7	3.4	3.1	3
Putrescine	3.5	3.2	3.6	3.2	2.5	2.3	3.4	3.2	3.1	3.4	4.1	3.32
Untreated control	21.3	22.7	28.3	29.3	8.2	9.3	11.8	10.8	31.2	30.2	21.2	22.2
LSD	0.4	0.5	0.6	0.4	0.36	0.3	0.37	0.4	0.51	0.3	0.4	0.5

Note: *Season I: 2015 and 2016 season; ** season II: 2016 and 2017 season.

3.3 Biochemical changes associated with induced resistance

Plant treatment with bio elicitors induced resistance in wheat plants against powdery mildew, rust and leaf spots with substantial reduction in disease severity. The induction of resistance was associated with many biochemical changes viz. increase in total phenols (mg catechol/g F.W.), hormonal stress as peroxidase activity (U g^{-1} of fresh weight), chitinase (nKat mg^{-1} of Protein) and total soluble protein (µg/mg FW) in the two wheat varieties, seasons and regions (Table 3 and 4). *Pseudomonas sp.* and *P. polymyxa* were more effective in increasing total phenols, peroxidase, chitinase and total soluble protein. At the same time, results clearly show that the chitosan and methyl jasmonate markedly increase the total phenols, peroxidase, chitinase and total soluble protein in both varieties.

Table 3 Chemical compositions in leaves of wheat plants, cv. Sakha grown under dry condition of Sinai and treaded with different products, 10 days after spraying, compared with normal condition of arable land in Behira

Treatments	Wheat Sakha 93 cv.							
	Total phenol ug^{-1} fresh weight (mg catechol/g F.W.)		Enzymes activities				Total soluble protein (µg/mg FW)	
			Peroxidase (U g^{-1} of fresh weight)		Chitinase (nKat/mg^{-1} of Protein)			
	Sinai	Behira	Sinai	Behira	Sinai	Behira	Sinai	Behira
Streptomyces griseus	19.2	20.2	18.7	19.4	4.2	4.9	20.1	20
Bacillus subtilis	27.1	28.2	20.1	20.7	5.1	6.6	24.1	25.1
P. polymyxa	36.2	38.1	29.3	29.9	7.6	9.8	35.1	36.2
Pseudomonas putida	33.1	35.2	25.2	25.7	7.2	9.2	31.1	31.5
P. fluorescens	31.8	32	21.3	22.9	6.8	7.2	29	30.7
Rhodotorula glutinis	19.1	19.9	18.3	18.7	5	5.7	25.1	25.9
Trichoderma harzianum	27.2	27.8	23.8	22.5	6.9	7.1	31.2	31.7
T. viride	26.8	27.2	21.9	23.9	6.1	6.8	30.2	29.1
Methyl jasmonate	34.1	33	27.4	28.6	7.3	7.8	32.2	31.2
Chitosan	40.1	39.1	26.1	26.8	7	7.3	33.1	35.1
Ascorbic acid	23.4	22.4	12.3	12.7	6	6.5	29.2	28.2
Putrescine	25	25.1	16.2	17.6	6	6.3	24	25
Untreated control	13.8	12.1	8.9	8	4.2	4	13.1	11

Table 4 Chemical compositions in leaves of wheat plants cv. Gemmiza grown under dry condition of Sinai and treaded with different products, 10 days after spraying, compared with normal condition in Behira

Treatments	Wheat Gemmiza 10 cv.							
	Total phenol ug^{-1} fresh weight (mg catechol/g F.W.)		Enzymes activities				Total soluble protein (µg/mg FW)	
			Peroxidase (U g^{-1} of fresh weight)		Chitinase (nKat/mg^{-1} of Protein)			
	Sinai	Behira	Sinai	Behira	Sinai	Behira	Sinai	Behira
Streptomyces griseus	19	20	18	19.8	4.1	4	20	18
Bacillus subtilis	26.3	27.2	20	21. 2	5	6.3	23.1	23.1
P. polymyxa	36	38	30	29	8	9	29	31.1
Pseudomonas putida	31	35.8	24	25.1	7	9	30	30.5
P. fluorescens	30.1	31	21	21.9	6.1	7	28	30
Rhodotorula glutinis	17.2	19	18	18	5	5.1	25	24.5
Trichoderma harzianum	27	26.3	22.2	21	6	7	31	30.6
T. viride	26.1	27.7	21.1	23	6	6.6	30	26.1
Methyl jasmonate	32.4	32.3	26	28.1	7	7.2	31.1	30.2
Chitosan	38.1	37.2	25.1	26.3	6.9	6.8	32.1	35
Ascorbic acid	21.4	21.2	12.1	12.3	6	6	29	28
Putrescine	23.3	24	16	16.3	5.3	6.1	23	24
Untreated control	12.1	13.4	8	12.3	4	5	13	15.5

3.4 Yield and Quality analysis

Yield of wheat is highly inter-related and both are significantly influenced by salt stress in Sinai than in normal soil (Table 5). According to presented data in Table 5, it could be noticed that the yield had a very significant influence. There was a highly significance difference between control and treated plants with different bio elicitors. Overall, the results suggest that the wheat yield was negatively influenced under saline soil that influence yield performance, meanwhile it's improved under treated conditions. Wheat plants treated with *P. putida*, *P. polymyxa* as well as methyl jasmonate, and Chitosan *resulted* in a significantly higher increase in the grain yield. The highest grain yields were obtained with the application of *P. polymyxa or R. glutinis the* gave the highest grain yields either in new reclaimed soils in Saini or arable land in Behira Governorate. These results are exact with both wheat cultivars as well as in both seasons (Table 5). It is worthy to mention that all treatments increased significantly the grain yield in comparison to untreated control. The results showed that flour, protein and gluten contents in wheat grains were highly inter-related and both are significantly influenced by salt stress as the result in (Table 6). *P. putida*, *P. polymyxa* resulted in a significantly greater increase of flour, protein and gluten contents in wheat grains under stress conditions. Methyl jasmonate, and Chitosan also give good results.

Table 5 Yield (ardab/fed.) of wheat grains, cvs Sakha and Gemmiza treaded with bio elicitors grown under dry condition of Sinai, compared with normal condition in Behira

Treatments	Sinai		Behira		Sinai		Behira	
	Season I	Season II	Season I	Season II	Season I	Season II	Season I	Season II
Streptomyces	18.7	20	18.4	20.7	18.9	17.3	19	21.1
Bacillus subtilis	19	19.9	21.6	22.1	19.5	20.3	21	20.3
P. polymyxa	21.5	22.1	22.3	22.8	20.9	22.3	22.4	22.4
Pseudomonas putida	21.1	21.2	22.3	23.6	20.7	21.8	22.2	21.5
P. fluorescens	20.6	19.8	20.4	22.3	18.8	21.2	20.6	20.3
Rhodotorula glutinis	20.4	20.7	22.4	22.8	18.5	21.2	19.2	19.7
Trichoderma harzianum	19.7	19.2	20.4	22.3	18.3	20.6	21.2	19.8
T. viride	17.6	18.5	19.8	19.9	19	20.5	20	19.7
Methyl jasmonate	19.7	19.9	20.6	20.6	19.9	20.6	21.2	19.9
Chitosan	20.5	20	20.6	20.9	19.5	20.3	21.3	20.3
Ascorbic acid	17.6	16.8	19.2	20	19.8	20.6	20.6	20.8
Putrescine	16	16.7	19.2	20	18.2	19.7	19.7	19.7
Untreated control	13.8	12.5	14.4	15.3	13.1	12.4	15.4	15.4
LSD	0.6	0.74	0.67	0.71	1.7	1.8	1.6	1.8

Note: * Season I: 2015 and 2016 season; ** season II: 2016 and 2017 season.

Table 6 Mean of flour content in wheat plants treaded with bio elicitors grown under saline soil in Sinai, compared with normal condition in Behira

Treatments	Flour yield* (%)				Flour protein* (%)				Gluten* (%)			
	Sinai		Behira		Sinai		Behira		Sinai		Behira	
	Saka 93	Gemmiza 10	Saka 93	Gemmiza 10	Saka 93	Gemmiza 10	Saka 93	Gemmiza 10	Saka 93	Gemmiza 10	Saka 93	Gemmiza 10
Streptomyces	12.6	12.2	13.6	13.1	11.7	11	11.9	11.2	27.3	26.4	29.4	28.3
Bacillus subtilis	13.1	12.2	14.2	14	11.4	11.2	11.7	11.2	30.8	29.3	32.4	31.2
P. polymyxa	14.3	14.1	15.8	15.1	12.5	12.1	12.7	12	32.4	30.4	33.4	33.3
Pseudomonas putida	15	14.9	15.1	15	12.4	12.4	12.8	12.1	33.4	31.3	36.4	33.5
P. fluorescens	13.7	13.4	14.4	14.2	12.1	12.6	12.8	12	29.7	28.4	30.1	30.6
Rhodotorula glutinis	14.2	14.3	14.6	14.2	12.2	12.7	12.8	12.1	31.3	29.3	31.3	32.3
Trichoderma harzianum	12.3	13.1	14.5	14.5	11.2	11.1	13.6	11.2	26.5	25.4	28.7	27.2
T. viride	12.3	12	13.8	12.2	11.3	11.5	12.5	12.2	24.5	23.4	27.8	24.4
Methyl jasmonate	14	13.1	13.9	13.5	11.2	11.1	12.7	12.2	27.8	26.3	30.4	29.3
Chitosan	14.7	12.3	13.7	13.9	12.3	11.1	12.2	11.2	28.9	27.3	29.3	29.5
Ascorbic acid	12.3	12.2	14.3	14	10.3	10.1	11.3	11.4	25.4	23.4	28.3	26.1
Putrescine	11.9	12.2	13.6	13.2	10.1	10.1	10.1	11.1	24.6	22.4	26.4	25.9
Untreated control	9.4	9.1	10.2	10	7.1	7.5	8.5	8.1	17.1	16.4	22.3	20.4

Note: * (% on dry weight basis).

4 Discussion

Wheat plants are exposing to biotic stress as pathogenic fungi that seriously reduce their growth and productivity. One of the direct results of climate changes in the host-pathogen-interaction is the plants resistance to pathogens. Reaction of plants to integrate environment and pathogen is strongly based on crop varieties, stage of growth and stress. Plant reaction to these stresses is multi-complex and includes different physiological and cellular adaptations and responses. Resistance to biotic stress has been well notarized in crop varieties through different type of defenses (Haggag, 2005; Pautasso et al., 2012; Haggag et al., 2014; Haggag et al., 2016). The plants defense strategy against biotic stress as diseases showed different types of stress proteins with different protective functions (Gianinazzi et al., 1970). The use of bioagents isolates as bio-elicitors in either dry climate stress in new reclaimed soils and arable land is possible to improve the tolerance of wheat plants against diseases. The applied approach could promote and encourage additive or synergistic inhibitory effects resulting from use of bioformulation of bio elicitors (Choudary et al., 2007 and Haggag et al., 2013). Application of bio-agents fungi and bacterium have been mentioned to support plant growth and elicit induced systemic resistance to plants against a range of pathogens when applied as seed treatments, soil drenches or foliar sprays. Field experiments confirmed the potentially of different bio elicitors in reducing the infection of diseases incidence and severity. The antagonistic efficiency has often been corresponding to production of different secondary metabolites, hormonal stress, biological and molecular characterization of microorganisms. These are useful as biocontrol agents or as producers of bioactive compounds, which are of noteworthy relationship for the echo-environment compatible agriculture. The potentially of a bio-elicitors to encourage resistance to disease has also been noted as a mechanism of controlling and management of plant diseases (Haggag et al., 2013. The effective species were *P. putida*, *P. polymyxa*, *R. glutinis* resulted in a significantly greater decrease in the diseases incidence as powdery mildew, rust and spots moreover, increased of total soluble protein, total phenols, chitinase and peroxidase in wheat plants grown in dry and normal regions. Application of methyl jasmonate, chitosan, an antioxidant in the form of ascorbic acid and a polyamine in the form of putrescein, significantly reduced diseases incidence and increased the total soluble protein, total phenols, chitinase and peroxidase. Strain of *Pseudomonas* spp. is known to produce HCN, and antibiotics (Jan et al., 2011). Fluorescent pseudomonads as *P. putida*, *P. polymyxa* and *P. fluorescens* are among the most effective rhizosphere rhizobacteria in reducing soil-borne fungal diseases. Several species of *Bacillus* including *B. subtilis* and *P. polymyxa* are widely known for their biocontrol and growth promotion abilities as they produce several antibiotics and phytohormones, solubilization of phosphate, releasing ammonia from nitrogenous organic matter (Hayat et al., 2010). Mechanisms of these bacterial species is mainly due to their potentially to produce antibiotics, lipopolysaccharides and iron regulated metabolites (Raaijmakers et al., 2002). Jasmonate, are known to play a great role in plant defense and protection via induced antifungal protein and proline –rich cell wall proteins as well as stimulate effect on secondary metabolite production including alkaloids, terpenes, phenolics and polyamines (Martin et al., 2002). In a previous research, a positive corroboration between bean rust disease inhibition and the over-accumulation of free and acid-soluble polyamine conjugates induced by a precursor of polyamines was obtained (Haggag, 2005). Bio elicitors increased plant growth, grain yield and flour, flour protein of wheat. The role of bio elicitors in plant growth promotion is widely known as equally important for overall plant growth. The quality characteristics specially flour, flour protein concentration and glutamine, are important one for the utilization the wheat bread and others use. These characteristics usually are effective by cultivar and interactions of cultivar with environment and diseases (Souza et al., 2004). In this study applying of bio-elicitors become more integrated into management control strategies in protection of wheat from biotic stress and permits a reduction in fungicide inputs. The results show that it could be possible to replace traditional chemical fungicides with bio-elicitors; it is safe for human health, environment and thus provided potential, economic and ecological value.

The results show that the used plant beneficial microorganisms as bio elicitors have improved the wheat tolerance to biotic stress under environmental changes in the new reclaimed lands under arid climate condition (Haggag, and Abdall, 2011).

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